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Family growth response to fishmeal and plant-based diets shows genotype×diet interaction in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The ability of rainbow trout to efficiently utilize plant-based diets for growth and the genetic variation for that trait have not been thoroughly examined. In this study, growth of a pedigreed population from the commercial Kamloop strain was assessed while feeding both plant-based and traditional fishmeal-based diets from initiation of feeding to termination of the growth trial at an average body weight of 600 g. Both fish oil (5.00%) and soybean oil (8.43%) were included in the plant-based diet, and only fish oil was used in the fishmeal diet (10.10%). Ninety-five (92 informative) full-sib families nested within 47 (46 informative) half-sib families were reared in a common environment. Parentage assignment was performed on approximately 1000 fish fed each diet using eight microsatellite markers chosen for non-duplication, a minimum of five alleles with no known null alleles, at least 50% heterozygosity, and unambiguous scoring. Progeny were assigned to parental pairs via two allocation programs, PAPA and FAP, to increase accuracy and to test efficiency. The fish fed the fishmeal/oil diet were approximately 8% larger than the fish fed the plant-based diet ($P<0.05$). A significant genotype×diet effect was detected. The variance component for this effect accounted for 5% of the sum of the variance components for all the random effects. The genetic correlation for growth on the two diets was 0.73±0.13, with a heritability of 0.31±0.07 on the plant diet and 0.32±0.07 on the fishmeal diet. We conclude that substantial genetic variation for utilizing plant-based diets containing soybean meal and oil exists in this widely used commercial rainbow trout strain.

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1. Introduction

The aquaculture industry has been criticized for the large volumes of fishmeal and fish oil used in feeds, particularly those for salmonids. The harvest of forage fishes for fishmeal has been relatively constant for several decades, and its availability imposes a major constraint on sustainable growth of global aquaculture production (Hardy, 2006). In addition, concerns about environmental impacts regarding effluent water quality and overexploitation of forage fishes as feed ingredients (Goldburg et al., 2002) have prompted increased examination of alternate diet formulations for aquaculture.

As an aquaculture business expands, so does the need for high-quality feeds. Of the seven major costs for trout farming – feed, labor, fish, energy, processing, marketing, and distribution – the price of feed can account for as much as 55% of variable production costs (Helfrich, 1997). High protein and energy feeds are required by salmonids since they utilize carbohydrates poorly. In order to meet nutritional requirements, most commercial feeds contain animal protein (mostly fishmeal), which is an expensive ingredient. Fishmeal-based diets are typically more expensive than plant protein-based diets. With the cost of high-quality fishmeal (65% protein) up to three times the price of soybean meal (Miles and Chapman, 2006), efforts have been made to reduce feed costs by using alternate, plant-derived ingredients (Gomes et al., 1995; Hardy, 1996; Sugiura et al., 1999; Carter and Hauler, 2000; Kissil et al., 2000; Barrows et al., 2007a).

Replacement of fish protein and oil in the diet by plant-derived protein and oil poses technical and biological challenges. Many plant byproducts contain lower protein levels and different balances of amino acids, often with limited amounts of essential amino acids (Adelizi et al., 1998). In addition, plant meals may contain anti-nutritional factors such as trypsin inhibitors, non-digestible carbohydrates, lectins, saponins, phytates, and, possibly, allergenic storage proteins (Salunkhe et al., 1992). Decreased palatability, which in turn could lead to decreased consumption, is a concern when fishmeal is replaced by plant meal (Davis et al., 1995; Stickney et al., 1996). In

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addition, the work by Geurden et al. (2005) demonstrates the preference of trout for fishmeal oil, and Francesco et al. (2004) deal with the fillet chemical composition and organoleptic characteristics of fish fed with plant diets.

In a previous study (Palti et al., 2006), we qualitatively compared the family affiliations among the largest and the smallest fish from 20 full-sib families fed traditional fishmeal- and plant gluten-based diets to evaluate genotype×diet interactions in the commercial "Spring" strain of rainbow trout. The results suggested that fish that grew faster when fed a fishmeal-based diet also grew faster when fed a fishmeal-free, gluten-based diet; i.e., there was no (or negligible) genotype×diet interaction in this strain. This finding raises the question of whether current commercial strains that exhibit superior growth can generally be expected to retain their improved performance when fed gluten-based diets.

In this study, we expanded our assessment of possible genotype×diet interactions in rainbow trout. Several changes were made in the plant-based diet in order to test a more commercially relevant diet of the future. Corn gluten meal, soybean meal, and a low level of wheat gluten were the primary protein sources, and the added oil was a 50% mixture of fish oil and soybean oil. We compared the growth response among 95 full-sib families fed the two diets from the widely used Kamloop strain of rainbow trout from Troutlodge, Inc. We randomly sampled 1000 fish from each diet group to estimate family mean and variance for size-at-age to calculate genetic correlations, and to quantitatively evaluate genotype×diet interaction.

2. Materials and methods

2.1. Fish stocks

The Kamloop strain from Troutlodge, Inc. (Sumner, WA, USA) that has been selected for improved growth for three generations using a best linear unbiased predictor (BLUP)-supported breeding value assignment program was the base population for this study.

2.2. Mating design and early rearing

One hundred and sixty (160) females were mated to 80 males on the basis of pedigree and breeding values using the following approach: Each male fertilized the eggs of two females, using the criterion that the parents of each cross can have no common grandparent. The parents were fin clipped for further DNA isolation and analysis. All the fertilizations occurred on the week of August 9–15, 2005. Once fertilization was completed, the process was followed by a common egg displacement procedure as described in Palti et al. (2006). The temperature×days of all lots was matched and they reached the "eyed" stage on the same day. Once egg development proceeded to the "eyed" stage, the family numbers were reduced to 95 full-sib families (approximately 9500 eggs) within a 47 half-sib group. The same number of eggs per family was then pooled from all families, mixed well, and randomly split into two groups that were incubated separately in hatching jars through hatching until the initiation of feeding.

At the start of feeding, the swim-up fry were placed into six 6000-l rearing tanks (three replicated tanks per diet) supplied with spring water (12 °C). Feeding was as described below. Bi-weekly sampling of weight was continued through grow-out. Fish were transferred from the rearing tanks to standard concrete grow-out raceways (three replicated raceways per diet) at an average weight of 25 g until harvest at approximately 600 g.

2.3. Diet formulation, and feeding regime

Two diets were formulated to be isonitrogenous and isolipidic (Table 1). The diets were manufactured at the Feed and Nutrition Laboratory of the Fish Technology Center in Bozeman, MT (U.S. Fish and Wildlife Service) for 0.5 to 3.0 mm pellet sizes. Additional 4.5 mm pellet sizes were produced by a commercial mill (Nelson and Sons, Murray, UT). Krill meal (5%) was added to the plant-based diet to increase palatability throughout the study. Astaxanthin was included in the 4.5 mm pellets.

Table 1
Composition of the two experimental diets

Plant diet		Fishmeal diet	
Ingredient	g/100 g	Ingredient	g/100 g
Krill meal	5.00	Fishmeal	63.14
Wheat gluten	7.04	Wheat flour	23.96
Corn gluten	34.57	Fish oil	10.10
Soybean meal	18.96	Lecithin	2.00
Wheat flour	14.43	Vitamin Premix #30[a]	0.50
Fish oil	5.00	Trace min #3[b]	0.10
Soybean oil	8.43	Stay-C	0.20
Lysine-HCl	1.47		
Methionine	0.45		
Taurine	0.50		
Dicalcium phosphate	2.55		
Vitamin Premix #30[a]	0.80		
Choline Cl	0.50		
Trace min #3[b]	0.10		
Stay-C	0.20		
Total	100.0	Total	100.0

Plant diet: 47.78% crude protein, 19.18% lipid, 3802 kcal/kg metabolizable energy. Fishmeal diet: 47.80% crude protein, 19.07% lipid, 3861 kcal/kg metabolizable energy.

[a] Contributed per kg of diet: vitamin A, 10,000 IU; vitamin D3, 720 IU; vitamin E, 530 IU; vitamin B12, 30 μg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; folacin, 13 mg; menadione sodium bisulfate, 25 mg; biotin, 1 mg; niacin, 330 mg.

[b] Contributed in mg/kg diet: zinc, 100; manganese, 70; iron, 3; copper, 2; iodine, 1.

Swim-up fry were fed manually at half-hour intervals through a 10-hour day. At the average weight of 25 g feeding was shifted to semi-automated feeders delivering pellets from a conveyor into the tanks at programmed intervals. Feed amounts were calculated using a proprietary feeding program and adjusted weekly based on biomass estimates. Feeding rates approximated satiation feeding. During the few days following the adjustment of feed amount to account for biomass gain, excess feed would remain in the feeders, while in the few days prior to the next weekly adjustment, all feed was consumed.

2.4. Sampling for parentage analysis

Once harvest weight was reached, a random sample of 1032 fish from each diet group (344 per raceway) was taken for measuring length and weight and for fin clipping. The fin clips obtained from these fish were stored in 100% ethanol until DNA extraction (Palti et al., 2002).

2.5. Markers and genotyping

Of the initial 2068 fin clips collected, 1996 were used for DNA extraction. The 72 not extracted were due to sample degradation or loss. After DNA extraction, purification, and quantification, samples were diluted to 12.5 ng/μl and used for PCR (polymerase chain reaction). Microsatellite multiplexes (Johnson et al., 2007) were used to decrease cost and laboratory time. However, two of the 12 markers were eliminated due to the presence of null alleles or genetic linkage with other markers in the multiplex. The 10 markers used were: *OMM5132*, *1008*, *5007*, *5047* and *5233* in multiplex 1 and *OMM5177*, *1051*, *1097*, *1088* and *1325* in multiplex 2. PCR conditions followed Johnson et al. (2007) with modified cycling times as follow. For multiplex 1: 95 °C for 10 min; 2 cycles of 94 °C for 1 min, 62 °C for 45 s, 72 °C for 2 min; 29 cycles of 94 °C for 1 min, 58 °C for 45 s, 72 °C for 2 min; 72 °C for 45 min; 4 °C for 1 h, and 12 °C hold. For multiplex 2, 95 °C for 10 min; 29 cycles of 94 °C for 1 min, 58 °C for 45 s, 72 °C for

2 min; 72 °C for 45 min; 4 °C for 1 h; and 12 °C hold. Amplifications were conducted on a Research DNA Engine thermal cycler (Model PTC 200, MJ Research, Waltham, MA, USA). PCR amplifications were verified on 3% agarose gels stained with ethidium bromide and diluted according to intensity. Three μl of each PCR product were diluted in 20 μl water, and 1 μl of the diluted product was mixed with 0.13 μl Rox-labeled 400 bp size standard and 12 μl HiDye-formamide. After denaturing, an ABI 3730 DNA Genomic Analyzer was used for fragment separation and visualization. The output data were analyzed using GeneMapper 3.5 software (ABI, Foster City, CA, USA).

2.6. Parental analysis

All parent and progeny genotypes were used as input for parental determination using the programs PAPA 2.0 (Duchesne et al., 2002) and FAP 3.5 (Taggart, 2006). The two programs were run simultaneously to increase accuracy and assess efficiency of parentage assignment. Progeny that were allocated differently between programs were evaluated and assigned manually to the correct full-sib families.

2.7. Statistical analysis

The frequency distribution of our random sampling per sire family and diet was evaluated to determine if survival was differentially affected by diet within families and if the overall distribution of sire families was different from the expected mean of 40 offspring. Paired *t*-test was used to compare distribution by diet within family and the Fit Ordinal Logistic function of JMP 5.0 was used to assess the deviation of the overall distribution from the expected mean.

Correlation between length and weight was estimated using JMP 5.0 (SAS Institute Inc., Cary, NC) to determine if body weight represents overall growth of the fish. Three regression analyses were produced: one for each of the two diets, and one for the entire population.

To evaluate phenotypic differences between the two treatments, we used SAS Proc Mixed, (SAS Institute Inc., Cary, NC) and the following equation to partition variance:

$$Y_{ijklm} = \mu + \mathrm{Diet}_i + \mathrm{Rway(Diet)}_{i(j)} + \mathrm{Sire}_k + \mathrm{Dam(Sire)}_{l(k)} + \mathrm{Dam(sire*diet)}_{l(kj)} + \varepsilon_{i(j)klm}$$

where Y_{ijklm} is fish size, μ is the grand mean; Diet_i is the variation due to the fixed effect of diet differences; $\mathrm{Rway(Diet)}_{i(j)}$ is the fixed effect of raceway within diet; Sire_k is the random effect of the *k*th sire, $\mathrm{Dam(Sire)}_{l(k)}$ represents the random effect of the *l*th dam within the *k*th sire, $\mathrm{Dam(sire*diet)}_{l(kj)}$ represents the random interaction effect between diet and the *l*th dam within the *k*th sire, and $\varepsilon_{i(j)klm}$ is the random error represented by the individuals within a full-sib family for each diet-within-raceway combination. Significance values of random effects then were determined by the size of the alpha value *P* using the Wald *Z* statistic for covariance parameters (SAS Institute Inc., 1999). If $P \leq 0.05$, differences were considered significant unless noted otherwise.

In addition, MTDFREML (Multi-Trait, Derivative-Free Restricted Maximum Likelihood), a genetic analysis program developed by Boldman and Van Vleck (1991), was used to estimate (co)variance components. In this analysis, growth on each diet was considered a separate trait. The genetic correlation between growth on the fishmeal-based diet and growth on the plant meal-based diet was evaluated using a missing value technique (S.D. Kachman, University of Nebraska, and L.D. Van Vleck, USDA-ARS, pers. comm.) with an animal model where the error correlation was set equal to zero because each animal was only tested on one diet. The correlation between the full-sib family means for body weight for the fishmeal (FM) and plant meal (PM) diets was evaluated as a Spearman rank correlation. The non-parametric Spearman correlation was chosen because of differences in the numbers of individuals representing each family (Altman, 1991). To evaluate the effect of differences in the numbers of individuals sampled from each family/diet, full-sib families with fewer than eight individuals per diet were excluded and the correlation was re-evaluated.

3. Results

3.1. Overall mortality rate

Dead fish were collected daily. The overall mortality rate of the two diet groups was similar. On the plant-based diet it was 4% and on the fishmeal diet 7%. In the raceways it was 1% on the plant diet and 3% on the fishmeal diet. No tank or raceway effects were observed.

3.2. Parentage assignment

All microsatellite markers used in the multiplex system proved informative. However, marker *OMM5132* was difficult to genotype due to the presence of alleles separated by only one base pair (Johnson et al., 2007). An unexpectedly high rate of genotyping errors was observed for marker *OMM5233*, reducing the success of pedigree assignment for the marker set. Data from these two markers were removed from parentage analysis. Data for the other eight markers then were used for parentage assignment.

A total of 1996 multilocus genotypes (1004 for fish fed the plant diet and 992 fed the fishmeal diet) were analyzed for parental assignment using both PAPA and FAP software packages. We manually eliminated 34 samples for which we could not obtain genotypes for at least seven of the eight markers. The two programs were unable to assign the same 113 individuals to any one set of parents. In addition, four other progeny, which could not be assigned to parents by PAPA were assigned by FAP. Another 29 progeny were assigned to different parents by each program. Data for these individuals were examined manually to determine their correct parental allocation. Eight progeny could not be assigned to a single parental pair (i.e., were ambiguous), and nine progeny could be assigned only to the sire. The remaining 12 progeny were divided equally between the two programs, with six assigned correctly by PAPA and six by FAP. Overall, we were able to assign parentage of both sire and dam to 1841 progeny (932 for plant protein and 909 for fishmeal), which were assigned to 92 full-sib families or 46 sire families.

All families were sampled in both diets and distribution by diet was very similar within sire families ($P > 0.45$ using paired *t*-test; Fig. 1), which implies that diet did not have a differential affect on survival within sire families, and our sampling was not biased. The overall distribution by sire families was significantly different from the mean expected number of 40 offspring per sire ($P < 0.0001$), which was

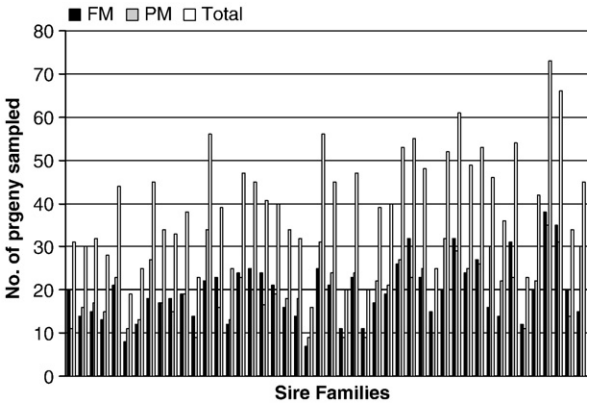

Fig. 1. Distribution frequency of the number of progeny sampled at random from sire families (two half-sib families each) shown for each diet and a combined total for each sire. The distribution was significantly different from the expected mean of 40 progeny per sire ($P < 0.0001$), but the paired *t*-test comparison of the distribution by diet within sire families was not significant.

caused by the expected variation of the random sampling process and differential survival between sire families.

3.3. Weight × length correlation

Weight by length regressions for each diet showed strong correlation between the two growth traits. We, therefore, used body weight as proximate of overall growth in our genotype × diet analyses. An r^2 value of 0.88 ($P<0.0001$) was shown for the plant diet, an r^2 of 0.80 ($P<0.0001$) was calculated for the fishmeal diet, and a combined r^2 of 0.85 ($P<0.0001$) for both diets.

3.4. Growth

The trout fed the fishmeal diet [mean = 645.5 (SD = 138.6 g)] were significantly heavier than fish fed the plant meal diet [589.8 (SD = 131.1 g)] ($P<0.05$). Significant raceway effects were detected by the fixed effects analysis (Fig. 2). A significant family × diet [Dam (sire * diet)$_{l(kj)}$] effect of 5% was detected by mixed model analysis (Table 2).

The heritability of growth was estimated by the MTDFREML animal model to be 0.31 ± 0.07 on the plant diet and 0.32 ± 0.07 on the fishmeal diet with a genetic correlation of 0.73 ± 0.13 for growth on the two diets.

An additional test of correlation between the full-sib family means for body weight for the fishmeal and plant meal diets was conducted to enable comparison of the results with our previous study (Palti et al., 2006). Although significant, the phenotypic correlation between the two diets was much weaker 0.28 ± 0.10 than the genetic correlation. When families with fewer than 8 samples on each diet were excluded, a total of 46 families within 29 sires were evaluated, which led to a correlation of 0.55 ± 013.

4. Discussion

4.1. Parentage assignment

Genetic improvements in aquaculture species have been reported with increasing frequency in recent years (Gjedrem, 2000). Results from commercial rainbow trout breeding programs have shown gains of approximately 15% per generation in selection for body size (James Parsons, unpublished data). Many aquaculture selection programs utilize a family-based mating design and benefit from the high fecundity of most aquatic species, and from external fertilization which enables simultaneous multiple matings and use of semen storage and cryopreservation for delayed fertilization. Full- and half-sib families possess the appropriate genetic relationships for estimating breeding values and genetic correlations among traits of interest (Falconer and Mackay, 1996). However, difficulty in marking small aquatic species often has necessitated the rearing of early developmental stages in individual family tanks, resulting in shared-tank effects among family members reared together (Winkelman and Peterson, 1994). Additionally, performance when families are reared separately is not necessarily representative of performance in mixed-family tanks (Herbinger et al., 1999). The use of genetic markers for assigning parentage and for pedigree analysis in "common garden" aquaculture experiments has become fairly common (O'Reilly et al., 1998; Fishback et al., 1999, 2002; Herbinger et al., 1999; Hara and Sekino, 2003; Sekino et al., 2003; Rodzen et al., 2004; Vandeputte et al., 2004; Palti et al., 2006) and allows evaluation of genotype × environment effects without confounding common-environment effects. However, the high cost of molecular genetics techniques needed to conduct these analyses has limited the use of "genetic tagging" by commercial breeders. Recently, we developed a microsatellite multiplex system for rainbow trout to effectively reduce the cost of reagents and time associated with pedigree allocation and genetic tagging in common garden breeding designs (Johnson et al., 2007).

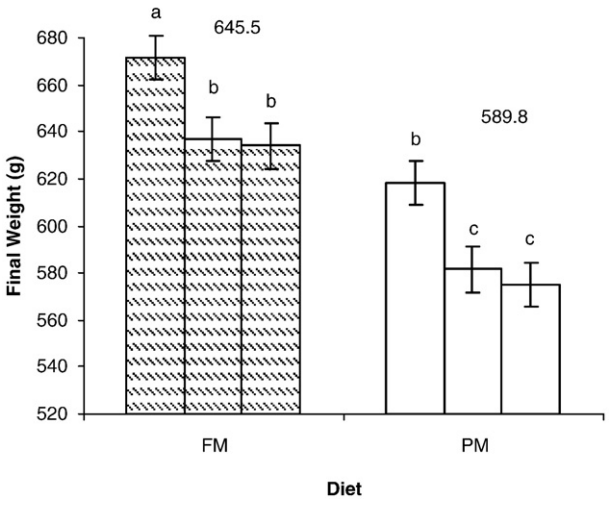

Fig. 2. Diet and tank effects on final body weight (g). Histogram bars indicate means of separate tanks. Final weights for the fishmeal diet (FM) are represented by dashed bars and for the plant diet (PM) by white bars. Standard errors are indicated. Different letters indicate significant differences of means between tanks and diets. Mean average weights are indicated above each set of histograms for the respective diets.

Table 2
Evaluation of random effects on body weight by mixed model analysis

Source	Variance component	Error	% Variance	*P* value
Sire	675	500	4	0.089
Dam(sire)	1103	565	6	0.018
Dam(sire) × diet	835	386	5	0.015
Residual	14,977	526	85	<0.001

The two parental assignment programs, PAPA 2.0 (Duchesne et al., 2002) and FAP 3.5 (Taggart, 2006), produced very similar results, agreeing in 98.4% of their parental-pair assignments. For the remaining 1.6%, FAP had an advantage over PAPA by identifying all possible parental pairs, whereas PAPA only identified one pair, even if ambiguity existed. Although FAP was not intended for this experimental design, it was useful for verification of PAPA's parental allocations. PAPA, which was used as the base program of this study, allowed restrictions on which parents, progeny, crosses, markers, and percent error to include. This permitted the user to limit the possible outcomes and only receive the output information desired. Using FAP, individual cross-mating restrictions are not allowed. Therefore, we used FAP to analyze the possible assignments from the database of all progeny to each individual parental cross separately.

4.2. Growth differences

A significant growth difference of approximately 8% was observed and was expected (Barrows et al., 2007a,b) between the fish fed the fishmeal diet and the fish fed the plant-based feed. It is possible to match the growth on fishmeal diet with a plant protein-based diet by using more expensive protein concentrates and avoiding soybean meal as partial protein source and the partial replacement of fish oil by soybean oil (Palti et al., 2006). Evidence of trypsin inhibitors, non-digestible carbohydrates, lectins, saponins, phytates and possibly allergenic storage proteins, have been discovered in soybean meal, all of which can hinder digestion and nutrient utilization in rainbow trout (Salunkhe et al., 1992). The plant-based diet in this trial, however, contained only 19.0% soybean meal which is below the generally regarded threshold of which performance declines. In addition, feed were processed using extrusion conditions shown to optimize performance of diets with high soybean meal levels (Barrows et al., 2007). Reduced feed consumption

due to a preference of trout for fishmeal oil (Geurden et al., 2005) or a possible imbalance in available amino acids could be the cause for the slight reduction in growth.

4.3. Genotype × diet interaction

The results of the quantitative genetic analysis in this study suggest a significant genotype × diet interaction, meaning that the families that grow faster on fishmeal-based diets are not necessarily the same families that would grow faster on the plant-based diet used in this study. When modeled without the genotype × diet [dam(sire × diet)] effect the dam (sire) and error sources of variance are inflated and the sire variance is unchanged (data analysis not shown). This indicates that the interaction effect is appropriate in reducing the error, and correctly assigning variance caused by dam(sire × diet), or genotype × diet, interaction.

These results differ from those of Palti et al. (2006), where family × diet interaction was not observed. The different findings may be explained by the addition of soybean oil and protein to the new diet and may partially be affected by the different rainbow trout strain used. It is also important to note that the experimental design of our previous study did not allow for quantified evaluation of the genetic correlation and the genotype × diet interaction.

The phenotypic correlation was much weaker than the strong correlation we saw previously between the family growth on fishmeal and plant gluten diets (Palti et al., 2006). This can be partially explained by the genotype × diet interaction we observed in this study, but the phenotypic correlation we observed in this study was also considerably lower than the genetic correlation. Exclusion of families with fewer than eight individuals in either diet reduced the number of families in the analysis by 50%. The subsequent increase in the magnitude of the correlation from 0.28 to 0.55 showed the phenotypic correlation to be sensitive to the number of individuals sampled per family. The genetic correlation, however, was less sensitive to sample size per family. It was calculated using the MTDFREML animal model, which accounted for a larger sample size per family, as it incorporated data from the full-sib and half-sib relatives into the family value. Indeed, Lynch and Walsh (1998) noted that genetic correlation is often greater than phenotypic correlation.

4.4. Implications for fish breeding

The Kamloop commercial strain of rainbow trout, previously selected for improved growth when fed standard fishmeal-based diets exhibited significant genotype × diet interaction. Thus, if plant protein/oil diets will be used in the future, selection strategies that account for that interaction should be considered. Breeding implications such as index selection, nucleus broodstock selection, or independent culling levels seem to be appropriate. Further investigation is needed to explore the genetic variation and to identify the genes involved in improved utilization of the specific plant diet we used. Studies that include screenings for quantitative trait loci and use of expression microarrays could help identify markers or genes of interest for targeted selection.

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